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Pyrenochaeta ingrati, a new species of food-borne coelomycetes

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Abstract – *Pyrenochaeta ingrati*, a new coelomycete isolated from a spoilt commercial apple juice in Japan, is described and illustrated. The new species differs from other species of *Pyrenochaeta* by the morphology of its conidiomatal setae, conidiophores and conidia. No pathogenicity for the apple fruits could be proven by an artificial inoculation experiment.

apple juice / coelomycete / *Pyrenochaeta ingrati* / taxonomy

Résumé – *Pyrenochaeta ingrati*, nouvelle espèce de coelomycete, isolée au Japon d'un jus de pomme commercial altéré, est décrite et illustrée. Cette nouvelle espèce diffère des autres *Pyrenochaeta* par la morphologie des *setae* pycnidiaux, des conidiophores et des conidies. Aucune pathogénicité n'a pu être démontrée par des inoculations artificielles.

INTRODUCTION

The occurrence of heat-resistant fungi in pasteurized fruit juices and other heat processed soft drink beverages is a main concern for the Japanese soft drink industry since the Food Regulatory Agency in our country has recently adopted the HACCP (Hazard Analysis Critical Control Points) system for the safe preparation of many foods. Insufficient processing is often contributing factor in foodborne-outbreaks. A variety of commercial pasteurized beverages which were undergoing spoilage were selectively surveyed for isolation of heat-resistants.

During the course of this survey, one isolate representing a hitherto undescribed species of *Pyrenochaeta* (Schneider, 1976, 1979, 1984; Sutton, 1980; Diederich, 1990; Sieber, 1995; Boerema et al., 1996; Grondona et al., 1997) was found which is herein described and illustrated. Thermal sensitivity of this fungus in Ringel solution was also briefly measured.

MATERIALS AND METHODS

The fungus was isolated once as a contaminant of a spoilage outbreak of commercial apple juice in Yokohama city, Japan. Single spore isolates were pre-

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pared to study cultural characteristics, morphology and heat inactivation test. Cultural characteristics were observed on potato-carrot (PCA) and potato-dextrose (PDA) agars after 14 days incubation at 25 °C under uv light (National UV tube, BL-B, 10 w) in a 12 h light/ 12 h dark cycle. Colour names were determined using Kornerup & Wanscher (1978). All measurements were made from water mounts.

A dried culture to serve as holotype has been deposited at the Natural History Museum and Institute, Chiba, Japan (CBM). An isotype is on deposited at the Collections du Laboratoire de Cryptogamie du Muséum National d'Histoire Naturelle, Paris, France (PC). The living cultures from the holotype have been deposited in Institute for Fermentation, Osaka (IFO) and the Siebold University of Nagasaki, Nagasaki (SUN), Japan.

An endpoint thermal death time (TDT) procedure was used for determination of heat inactivation. Ringel solution was used as a medium. Portions (1 ml) of conidial suspension were heated in sealed TDT tubes for four time intervals (1, 5, 10, and 15 min) at each of three temperatures (55, 60, and 65 °C). Five tubes were heated at each condition. Heated tubes were cooled and the contents cultured at 25 °C for at least 4 weeks.

Pathogenicity of the isolates from apple juice was tested for apple fruits. Apples were washed in detergent, rinsed in distilled water, and surface-sterilized with 70% alcohol. The inoculum from a 21 day-old PDA culture of the fungus was introduced into the flesh through scalped wounds in the sides of the fruits. Fruits were held in moist chambers at 25 °C for 45 days.

TAXONOMY AND RESULTS

Pyrenochaeta ingrati Udagawa & Toyazaki, sp. nov. Figs. 1, 2.

Coloniae in PCA effusae, floccosae, griseo-virides vel atrovirides; conidiomata numerosa; reversum obscure viride vel viridi-griseum. Conidiomata (pycnidia) dispersa, vulgo solitaria, brunnea vel fere nigra, globosa vel subglobosa, unilocularia, (65-)80-120 × 70-110 µm, ostiolata, setosa, cum colo papillato; peridium tenue, brunneum vel atrobrunneum, membranaceum, duobus stratis textura angulari compositum, stratum exterius ex cellulis angulatis brunneis 4-8 µm diam compositum, stratum interius ex cellulis complanatis hyalinis. Setae olivaceo-brunneae, rectae, 1-6-septatae, 14-60 × 2-4 µm, fere leves, apice acutae vel rotundatae. Conidiophora hyalina, simplicia vel ramosa, filiforma, usque 30 µm longa, 1.5-4 µm diam, septata. Cellulae conidiogenae hyalinae, simplices, phialidicae, enteroblasticae, ampulliformes vel doliiformes. Conidia hyalina, cylindrica vel ellipsoidea, 2.8-4.8 × 1.2-1.6 µm, levia, primo guttulata.

Holotypus: CBM-SUM 3166, colonia exsiccata in cultura ex succo pomaceo putrido, Yokohama, in Japonia, 17 Apr. 2000, a S. Udagawa isolata et ea CBM conservata.

Etymology: from Latin, *ingratus* = disagreeably, referring to the spoilage incidence in a commercial apple juice.

Colonies on PCA growing rapidly, attaining a diameter of 48-50 mm in 14 days at 25 °C, deeply floccose from the development of abundant aerial hyphae, consisting of a thin basal mycelium bearing numerous conidiomata, Greyish

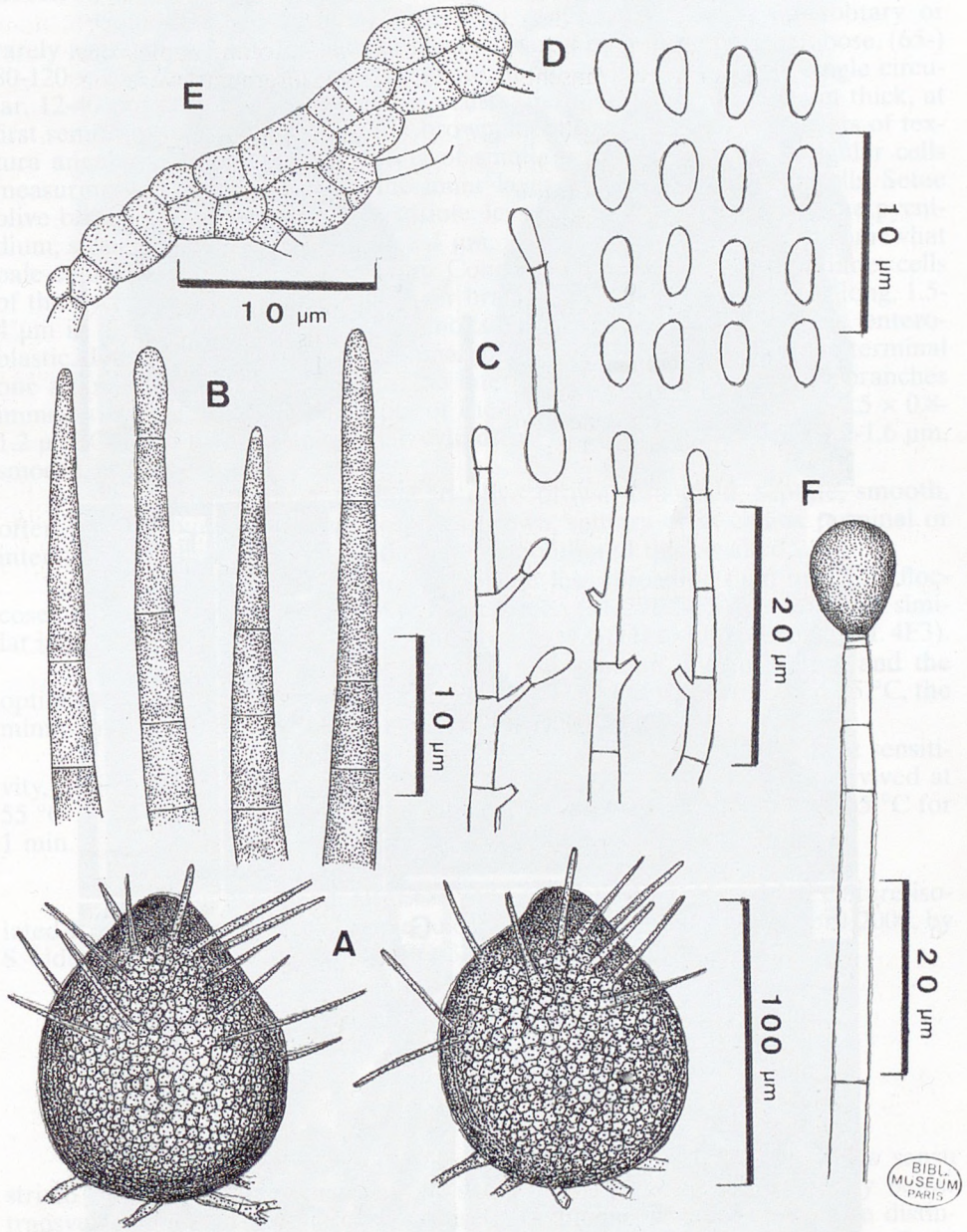

Fig. 1. *Pyrenochaeta ingrati*. A. Pycnidia. B. Setae. C. Conidiophores and conidiogenous cells. D. Conidia. E. Initial of pycnidium. F. Chlamydospore.

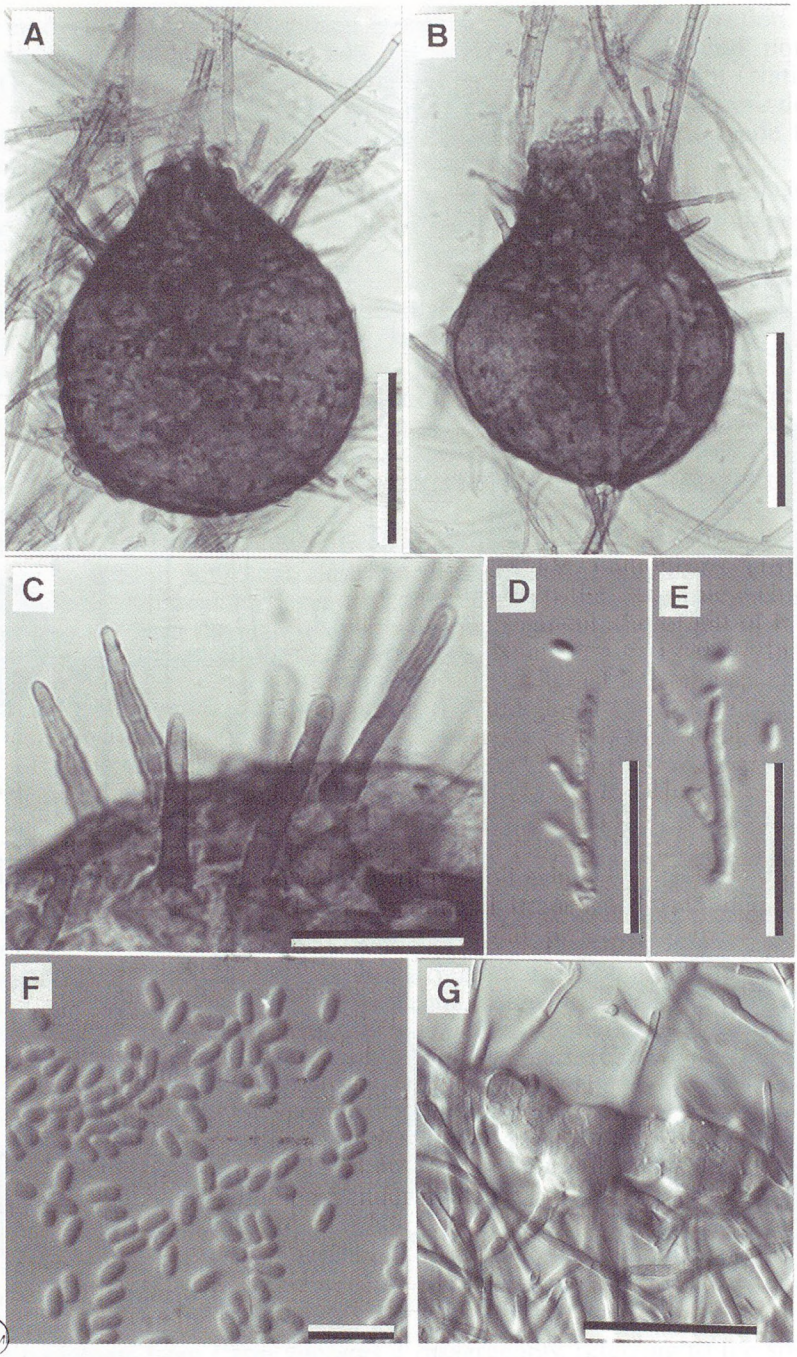

Fig. 2. *Pyrenochaeta ingrati*. A, B. Pycnidia. C. Part of pycnidial wall, showing setae. D, E. Conidiophores and conidiogenous cells. F. Conidia. G. Initial of pycnidium. Scale bars: A, B = 50 μm; C, D, E, G = 20 μm; F = 10 μm.

Green (M. 28E5) to Dark Green (M. 29F3); margins thin, narrow; reverse Dull Green to Greenish Grey (M. 27E3-F2).

Conidiomata (pycnidia) superficial or immersed, scattered, solitary or rarely aggregated, brown to almost black, unilocular, globose to subglobose, (65-) 80-120 × 70-110 µm, setose; neck papillate, about 20 µm high, with a single circular, 12-40 µm diameter ostiole at the centre; peridium thin, about 6 µm thick, at first semitransparent, brown to dark brown, membranaceous, of two layers of textura angularis; outer layer consisting of small, brown, thick-walled angular cells measuring 4-8 µm in diameter; the inner layer of hyaline, flattened cells. Setae olive brown, frequent around the ostiole, less covered over the rest of the pycnidium, straight, 1-6-septate, 14-60 × 2-4 µm, almost smooth, tapering to somewhat pale-coloured, pointed or rounded tip. Conidiophores formed from the inner cells of the pycnidial wall, hyaline, simple or branched, filiform, up to 30 µm long, 1.5-4 µm in diameter, septate. Conidiogenous cells hyaline, simple, phialidic, enteroblastic, determinate, integrated, terminal and often becoming lateral, the terminal one ampulliform, 6.5-8 × 1.5-2 µm, the lateral one arising as very short branches immediately below transverse septa of the conidiophore, doliiform, 1.5-2.5 × 0.8-1.2 µm. Conidia hyaline, unicellular, cylindrical to ellipsoidal, 2.8-4.8 × 1.2-1.6 µm, smooth, at first guttulate.

Vegetative hyphae hyaline to olive brown, branched, septate, smooth, often forming bundles. Chlamydospores brown, solitary or in chains, terminal or intercalary, swollen to 8.5 µm in diameter, smooth and thick-walled.

Colonies on PDA growing somewhat less spreading than on PCA, floccose to fasciculate, Greyish Green to Dark Green (M. 29E5-F5); conidiomata similar in production to PCA; reverse Light Brown (M. 5D4) to Olive Brown (M. 4F3).

Other characteristics for growth: the minimum, the maximum and the optimum temperatures are below 5 °C, 35 °C and 25 °C, respectively; at 25 °C, the minimum Aw is 0.88; and pH range 3-10.

Inactivation temperature: As results of the TDT method for heat sensitivity, conidia of this fungus, which were suspended in Ringel solution, survived at 55 °C for 15 min and at 60 °C for 1 min, but survivors were only few at 65 °C for 1 min.

Pathogenicity for the apple fruits: none.

Specimen examined: HOLOTYPE, CBM-SUM 3166, a dried culture isolated from spoilt commercial apple juice, Yokohama-shi, Japan, 17 April 2000, by S. Udagawa. Ex-holotype strains IFO 33278 and SUN.

DISCUSSION

According to Sutton (1980), conidiogenous cells in *Pyrenochaeta* sensu stricto are described: arising as very short lateral branches immediately below transverse septa of long filiform multiseptate conidiophores. This feature distinguishes *Pyrenochaeta* de Not. from setose pycnidial members of *Phoma* Sacc. (Schneider, 1976, 1979, 1984). Thus our fungus was better placed in the genus *Pyrenochaeta*.

In Japan, the two known species clearly belong to the genus: *P. lycopersici* Schneider & Gerlach and *P. oryzae* Shirai ex I. Miyake (Miyake, 1910; Morita et al., 1975; Punithalingam, 1980). *P. ingrati* is easily distinguished by pycnidial and

conidial characteristics; *P. lycopersici* has large pycnidia with long setae (100-150 (-300) μm long), larger conidia (3-8 μm or 5-7 μm long) and microsclerotia (Schneider and Gerlach, 1966; Sutton, 1980); *P. oryzae* also differs in producing large pycnidia with long setae (50-140 μm long) and fusiform, longer conidia (4-6 μm long).

Two other species of *Pyrenochaeta*, *P. nipponica* Hara and *Pyrenochaeta* sp., which are pathogenic to rice plants were reported from Japan, although the descriptions of both species were uncertain in the presence of *Pyrenochaeta* type of conidiophores and conidiogenous cells (Hara, 1959; Mori et al., 1964). However, the differences in pathogenicity are definite, because the new species was not pathogenic to rice plants in our repeated inoculation experiments (unpublished data).

Pyrenochaeta mali M.A. Smith, the causal agent of *Pyrenochaeta* rot of apple fruits, was described by Smith (1963) from the USA (Smith, 1963; Snowdon, 1990). This fungus is characterized by brown to black, pycnidia with short, 1-5-septate setae, and hyaline, 1-celled, oblong-ovoid, 5-7.5 × 2.25-3.75 μm conidia. From the original description, conidiogenesis of this fungus can not be ascertained, although conidiogenous cells in the photographic illustrations seem to be *Phoma*-like. The new species apparently differs from *P. mali* by its smaller conidia. To further distinguish both species, we had confirmed by our artificial inoculation test of *P. ingrati* that no rotting signs could be found in the apple fruits.

The evidence of its no pathogenicity for apple fruits and heat sensitivity suggested that *P. ingrati* possibly represented one of survivors in contamination which may come from outside sources during post-pasteurization handling and processing of soft drinks.

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